## REMARKS

The Applicants have carefully considered this application in connection with the Examiner's Action and respectfully request reconsideration of this application in view of the foregoing amendment and the following remarks.

In response to the present office action the Applicants have amended Claims 1, 10, 15 and 24 and canceled Claims 2-3, 5-6, 9, 14, 16-20 and 22. Accordingly, Claims 1, 4, 7-8, 10-13, 15, 21, and 23-36 are currently pending in the application.

### I. Rejections of Claims Under 35 U.S.C. §112 Second Paragraph

The Examiner has maintained the rejection of Claims 1-14, 16-18 and 23-36 under 35 U.S.C. §112, second paragraph, for failing to particularly point out and distinctly claim the subject matter that the Applicants regard as the invention. Specifically, the Examine maintains that the term "R1 includes organic substituents," is vague and indefinite.

In response, the Applicants have amended Claims 1, 10 and 24 to clearly define R1.

Therefore, the Applicants and respectfully request that the 35 U.S.C. §112 second paragraph rejections of Claims 1, 4, 7-8, 10-13, 15, 21, and 23-36 be withdrawn.

### II. Rejection of Claims under 35 U.S.C. §103

The Examiner has maintained her rejections of Claims 1-14, 16-18 and 23-36 under 35 U.S.C. §103(a) as being unpatentable over Aime *et al.*, Chem. Commun., 1999, 1047-48 ("Aime") in view of Ward *et al.*, Journal of Magnetic Resonance, March 2000, 143, 79-87 ("Ward") and Dunard *et al.* J. Am. Chem. Soc., February 2000, 122, 1506-12 ("Dunard").

The Applicants respectfully maintain that the claimed invention is not obvious in view of the foregoing combined references, and that this combination fails to establish a *prima facie* case

of obviousness of Claims 1, 4, 7-8, 10-13, 15, 21, and 23-36.

In maintaining her rejections, the Examiner asserts that the difference between the instant invention and the prior art is that the latter do not specifically state that the water molecule has a $\Delta\omega \bullet \tau_M \geq 1$ and a $\Delta\omega \geq 6$ ppm. The Examiner reasons that structurally similar contrast agents would inherently have the same or similar properties. The Examiner further states that it is unclear why or how the compounds of the instant invention, which are encompassed by the prior art, lack the same properties thereof. The Examiner also states that the cited references, Aime, Ward and Dunard, were combined because they disclose a contrast agent comprising a tetraazacyclododecane structure. In an earlier Office Action mailed November 10, 2003 the Examiner stated that all of the references are directed to DOTA-lanthanide complexes and therefore are considered to be in the same field of endeavor.

The Applicants respectfully maintain that the asserted combination of Aime in view of Ward and Dunard fail to teach or suggest all of the elements of the invention recited in independent Claims 1, 10 and 24.

The Applicants have amended Claims 1, 10 and 24 to further clarify that the present invention is directed to chemical exchange saturation transfer (CEST) contrast agents, and their method of use. The CEST contrast agents of the present invention have particular structures that allow the transfer of a saturating magnetization from a water molecule bound to a paramagnetic metal ion-macrocylic complex to bulk water. As pointed out in paragraph [0041] of the specification, certain paramagnetic metal ion-macrocylic complexes are conducive to the bound water molecule having an exchange rate sufficiently slow to produce an observable magnetic resonance signal. The paramagnetic metal-ion macrocylic complexes must also alter the shielding around the protons of the bound water molecule so as to have a chemical shift that is different from the protons of bulk water. These requirements are particularly pointed out and distinctly claimed in amended Claims 1, 10 and 24 by reciting that the bound water molecule has a $\Delta\omega \bullet \tau_M \geq 1$ and a $\Delta\omega \geq 6$ ppm.

As further explained in the declaration by Dr. Dean Sherry, submitted along with the

present response, it is by no means obvious which combinations of paramagnetic metal ion and tetraazacyclododecane ligand structures will provide an environment where a bound water molecule can have these properties. Amended Claims 1, 10 and 24 are directed to those tetraazacyclododecane ligands having pendent arms R, R', R'' and R''' and having a general formula of $-CR_1H-CO-NH-CH_2-R_2$, with $R_1$ and $R_2$ as defined in these Claims. These tetraazacyclododecane ligands in combination with certain paramagnetic metal ions can provide a suitable environment for the bound water molecule.

In contrast, Aime discloses that the pendant arms of compounds L3a or L3b are all: $-CH_2-CO-NH-CH_2-CH_2-COX$ (where X = OEt or $O^-$, respectively). The pendant arms of L3a or L3b do not fall within the scope of the pendant arms recited in the claimed inventions because a $-CH_2-CH_2-COX$ group (where X = OEt or $O^-$) is attached to the nitrogen atom in the pendant arms. In Claim 1 for instance, when $R_1$ is H and $R_2$ is -COOEt or $COO^-$, then at least one of the pendant arms R, R', R'' and R''' is $-CR_1H-CO-NH-CH_2-COOEt$ or $-CR_1H-CO-NH-CH_2-COO^-$, respectively.

As explained in the declaration by Dr. Sherry, introducing an additional $-CH_2-$ between the nitrogen atom and the $R_2$ group of a pendant arm would unpredictably alter the structure and properties of the ligand-metal-water complex. Consequently, one of ordinary skill in the art could not readily predict whether or not a bound water molecule would have the requisite properties recited in Claims 1, 10 and 24. Nor would one of ordinary skill in the art have motive to remove the additional $-CH_2-$ in Aime's compounds L3a or L3b to arrive at the claimed invention because none of Aime, Ward or Dunard provide any teaching or suggestion as to what structural attributes of pendant arms are conducive to a CEST agent. This stands to reason because Aime is interested in examining the luminescence properties of Eu-heptadentate and octadentate ligands, $L^{1a}$, $L^{1b}$, $L^{2a}$, $L^{2b}$, $L^{3a}$ and $L^{3b}$, or the relaxivity of Gd complexes of these same ligands. As pointed out in the previous response filed on February 24, 2004, there is no recognition by Aime that such complexes could be suitable as CEST contrast agents.

Similarly, Dunard only discloses the use of DOTA (pendant arm: $-CH_2-COOH$) and

DOTAM (pendant arm: $-CH_2-CO-NH_2$) ligands, neither of which have pendant arm structures that fall within the scope of the pendant arm structures recited in Claims 1, 10 and 24. In addition, as pointed out in the previous response, Dunard does not consider DOTA or DOTAM for use as a CEST contrast agent. Dunard examines $^{1}H$ and $^{17}O$ NMR linewidths of bound water for $[Eu (DOTAM)(H_2O)]^{3+}$ because the bound water NMR signal of $[Gd(DOTA)(H_2O)]^-$ is not observable (pp. 1507; column 1). Furthermore, Dunard's magnetization transfer experiments on $[Eu(DOTAM)(H_2O)]^{3+}$ involve saturating the magnetization of cyclic protons to study ring inversion or pendant arm rotation (FIGURE 5 and pp. 1509; column 2). Dunard, therefore, does not teach or suggest that $[Eu (DOTAM)(H_2O)]^{3+}$, can serve as a CEST contrast agent. As further explained in the declaration of Dr. Sherry, Eu-DOTAM is a poor candidate as a CEST contrast agent.

Moreover, Dunand's stated goal is to understand the mechanism of water exchange in conventional Gd-based contrast agents in order to design new Gd-based contrast agents (pp. 1506; column 2). As explained in paragraph [0003] of the present application, gadolinium containing contrast agents are thought to achieve contrast by a paramagnetic relaxation effect of the metal-ion to shorten the bulk water relaxation time via rapid exchange of the metal ion's inner-sphere water molecules with bulk solvent. Thus, to design new Gd-based contrast agents Dunard's teaching are directed to providing a ligand that promotes rapid exchange of the metal ion's inner-sphere water molecules--the opposite of what is desirable for the design of a CEST contrast agent of the present invention.

Ward does not disclose a contrast agent comprising a tetraazacyclododecane structure, and certainly not a tetraazacyclododecane ligand having the pendant arm structure recited in Claims 1, 10 and 24. For instance, Gd-DPTA, gadolinium diethylene triaminepentaacetic acid mentioned on page 79 of Ward is not a tetraazacyclododecane ligand. None of the compounds listed in Table 1 of Ward are tetraazacyclododecane ligands. If such a teaching of tetraazacyclododecane ligands does exist, the Applicants would appreciate it if the Examiner could point out where this can be found in Ward.

As pointed out previously, Ward specifically rejects metal-based contrast agents because of several perceived drawbacks (page 79). As such, Ward does not teach or suggest that metal-ligand complexes could be used as CEST contrast agents, let alone what pendant arm structure of a tetraazacyclododecane ligand, and which metal ions, provided the appropriate environment for a bound water molecule.

In summary, because the asserted combination of Aime in view of Ward and Dunard fails to teach or suggest all of the elements of the inventions of Claim 1, 10 and 24, the Examiner cannot establish a *prima facie* case of obviousness of dependent Claims 4, 7-8, 11-13, 15, 21, 23 and 25-36, which includes all the elements of their respective independent claims.

In addition, the Applicants respectfully maintain that the asserted combination of Aime in view of Ward and Dunard fails to establish a *prima facie* case of obviousness because this combination is improper.

The Examiner stated in the previous office action mailed February 3, 2004 that both Dunard and Ward disclose the evaluation of CEST in various compounds. The Examiner also stated that all of the references are directed to DOTA-lanthanide complexes and therefore are considered to be in the same field of endeavor. However, as explained above, the evaluation of CEST contrast agents was not the objective of the studies by Aime and Dunard. Additionally, Ward rejected the use of metal-based contrast agents, and contrary to the Examiner's assertion, does not disclose a contrast agent comprising a tetraazacyclododecane ligand structure.

The Applicants maintain that the combination of Ward with either of Aime and Dunard is improper because a person having ordinary skill in the art would not be motivated to find or add to Aime or Dunard the teachings and suggestions of Ward, because Ward rejects the use of metal-based contrast agents and Aime and Dunard are not directed to the development and use of CEST contrast agents. As noted above, Dunard is directed to understanding the structure, rate and mechanism of water exchange in order to design new Gd-based MRI contrast agents. Aime is directed to determining the pH dependence of the luminescence of certain Eu-ligand complexes, and the $^{1}H$ relaxivity of certain Gd-ligand complexes and in the presence and

absence of hydrogen carbonate.

In view of the foregoing remarks, the cited references do not support the Examiner's rejection of Claims 1, 4, 7-8, 10-13, 15, 21, and 23-36 under 35 U.S.C. §103(a). The Applicants therefore respectfully request the Examiner to withdraw the rejection of these claims.

## III. Conclusion

In view of the foregoing amendments and remarks, the Applicants now see all of the Claims currently pending in this application to be in condition for allowance and therefore earnestly solicit timely a Notice of Allowance for Claims 1, 4, 7-8, 10-13, 15, 21, and 23-36.

The Applicants request the Examiner to telephone the undersigned attorney of record at (972) 480-8800 if such would further or expedite the prosecution of the present application.

Respectfully submitted,

HITT GAINES, P.C.

Charles W. Gaines
Registration No. 36,804

Dated: 7/7/04

P.O. Box 832570
Richardson, Texas 75083
(972) 480-8800

DOCKET NO. UTAD-0001 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: A. Dean Sherry, *et al.*

Serial No.: 10/001,858

Filed: November 20, 2001

Title: PARAMAGNETIC METAL ION-BASED MACROCYLIC MAGNETIZATION TRANSFER CONTRAST AGENTS AND METHOD OF USE

Grp./A.U.: 1616

Examiner: Dameron Levest Jones

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231

on July 7, 2004
(Date of Deposit)

Elizabeth Schumacher
(Signature of Person Depositing)

Elizabeth Schumacher
(Printed Name of Person Depositing)

Sir:

## DECLARATION OF DEAN SHERRY IN ACCORDANCE WITH 37 C.F.R. § 1.132

The undersigned, Dean Sherry, a United States citizen having an address of 6934 Spanky Branch Drive Dallas, Texas 75248 declares the following:

1. I am a graduate of the University of Kansas State University, Manhattan, Kansas with a Doctor of Philosophy (PhD) degree in Chemistry.

2. I am presently employed as Professor of Chemistry by the University of Texas at Dallas (50% time) and Professor of Radiology by the University of Texas Southwestern Medical School at Dallas (50% time). I have been engaged in the design and development of Magnetic Resonance contrast agents for about 25 years.

3. I am an inventor of the present application, have read and understand the Examiner's Actions mailed November 10, 2003 and May 18, 2004, and would like to clarify issues raised by the Examiner in the Action regarding the general teaching of contrast agents as presented in Aime *et al.*, Chem. Commun., 1999, 1047-48 ("Aime"), Ward *et al.*, Journal of Magnetic Resonance, March 2000, 143, 79-87 ("Ward") and Dunard *et al.* J. Am. Chem. Soc., February 2000, 122, 1506-12 ("Dunard"), and their putative relationship to the design of the CEST contrast agents of the present invention.

4. The tetraamide-tetraazacyclododecane ligands of the present invention, in combination with certain paramagnetic metal ions, change the shielding around the proton atoms or a bound water molecule so that the protons have a $^1H$ NMR signal that is substantially shifted from that of bulk water (this is the term $\Delta\omega$). It is also known that tetraazacyclododecane ligands with tetraamide side-chains give lanthanide complexes that have extremely slow water exchange kinetics, in many cases so slow that irradiation of the $^1H$ NMR signal of the $Ln^{3+}$-bound water molecule results in transfer of saturated spins to the bulk water $^1H$ NMR signal. This is the basis of the present invention. Although we known that tetraamide side-chains impact slow water exchange kinetics to the resulting $Ln^{3+}$ complexes, it is still not possible to *a priori* predict the water exchange rates just by knowing the chemical structure of the appended amide side-chains. We know today that water exchange in these systems dramatically depends upon 1) the ionic radius of the central $Ln^{3+}$ cation (Zhang, S.; Wu, K.; Sherry, A.*D.; J. Am. Chem. Soc.* **2002**, *124*, 4226.) and 2), as reported by others, the bulkiness of the side-chain R groups (Aime, S.; Barge, A.; Batsanov, A.S.; Botta, M.; Castelli, D.D.; Fedeli, F.; Mortillaro, A.; Parker, D.; Puschmann,

H.; *Chem. Commun.* **2002**, 1120.). These data, however, were not known at the time of the present invention. Even today, it is difficult (arguably impossible) to predict whether any given tetraamide side-chain complex will display slow enough water exchange to meet the condition $\Delta\omega \bullet \tau_M \geq 1$. For example, $Eu^{3+}$ complexes formed with a fully substituted tetraazacyclododecane macrocycle having pendant arms, $-CH_2-CO-NH-CH_2-CO_2^-$, $-CH_2-CO-NH-CH_2-CO_2-CH_2-CH_3$ and $-CH_2-CO-NH-CH_2-PO_3(CH_2-CH_3)_2$ all show a $Eu^{3+}$-bound water resonance near 50 ppm and display slow enough water exchange to meet the criteria, $\Delta\omega \bullet \tau_M \geq 1$, yet the $Eu^{3+}$ complex formed with the corresponding tetra-substituted $-CH_2-CO-NH-CH_2-PO_3^{2-}$ groups does not. Thus, the first three may be used as paramagnetic CEST agents while the later complex does not produce any effect. Furthermore, in my opinion, one skilled in the art cannot predict whether the compounds, $L^{3a}$ or $L^{3b}$, disclosed in Aime *et al.*, Chem. Commun., 1999, 1047-1048 having pendant arms of $-CH_2-CO-NH-CH_2-CH_2-COOEt$ and $-CH_2-CO-NH-CH_2-CH_2-COO^-$ would meet this exchange criteria or not. Moreover, although today one may predict that $\Delta\omega$ would be near 50 ppm in the $Eu^{3+}$ complexes formed with these ligands, <u>one can only evaluate the true $\Delta\omega$ if water exchange is slow enough to actually detect the $Eu^{3+}$ bound species by high resolution $^1H$ NMR spectroscopy</u>. It is also my opinion that one skilled in this art, can not predict whether or not water exchange will be slow enough in these complexes to meet the exchange criteria $\Delta\omega \bullet \tau_M > 1$. This can only be determined experimentally by measuring the actual water exchange rate for the $Eu^{3+}$ complexes. If one assumes that the bound water lifetime of $[EuL^{3b}]^-$ is similar to the value reported in Aime *et al.* 1999 for $[GdL^{3b}]^-$ are similar (19 μs), then $\Delta\omega \bullet \tau_M$ for this complex would be 0.057 at 60 MHz (the common clinical imaging field strength), 0.095 at 100 MHz, 0.19 at 200 MHz, and 0.38 at 400 MHz. Thus, $[EuL^{3b}]^-$ would not be covered by the present invention because we are claiming only complexes having $\Delta\omega \bullet \tau_M \geq 1$. Given these estimates and the fact that no CEST studies have been reported on these compounds since the Aime publication in 1999, I predict that water exchange is too fast to detect a CEST effect in these complexes.

The only two macrocyclic ligands discussed by Dunard *et al.* (J. Am. Chem. Soc., February 2000, 122, 1506-1512) were DOTA and DOTAM having pendant arms of $-CH_2-COOH$ and $-CH_2-CO-NH_2$, respectively. Complexes of $EuDOTA^-$ and $EuDOTAM^{3+}$ are also not claimed in the present invention. Neither the DOTA nor DOTAM structure are covered by the claims. Furthermore, the data presented by Dunard *et al.*, do not teach anything about the potential of using such complexes as CEST agents. Although it was not made clear in that paper, the data presented in Table 1 were measured on GdDOTAM not EuDOTAM. These data are further complicated by the presence of two coordination isomers present in solution for this system (designated the M-isomer and the m-isomer). The reported $\tau_M$ values for GdDOTAM (not EuDOTAM) are 120 μs for the M-isomer and 3 μs for the m-isomer. In practical terms, the $\tau_M$ value important for CEST considerations would be the weighted average of these values, each weighted by the fraction of the two isomers present. This weighted average $\tau_M$ value is reported on the bottom of p. 1508, second column, as 14 μs (the inverse of $7x10^4$ $s^{-1}$). Furthermore, it is then assumed on p. 1509 that water exchange would be somewhat faster than this for the corresponding $Eu^{3+}$ complex. Hence, using the same assumptions as outlined above, the product, $\Delta\omega \bullet \tau_M$ would be much, much smaller than 1 at any common magnetic field strength, and therefore, EuDOTAM would not be covered by the present invention. In fact, one can argue that Dunard, et al. not only does not teach one skilled in the art about the potential of using EuDOTAM as a paramagnetic CEST agent, it in fact teaches just opposite. Dunard, *et al.* further illustrates that one cannot predict that $\Delta\omega \bullet \tau_M$ will be $\geq 1$ just based upon structure.

Turning to Ward, I can find no discussion of tetraazacyclododecane ligand structures. Therefore this reference is not informative as to what combinations of pendant arms structures in a tetraazacyclododecane ligand and paramagnetic metal ions are appropriate for the design of CEST contrast agents.

5. The undersigned declares that all statements made herein of his own knowledge are true, and all statements made on information are believed to be true; and furthermore, that the statements were made with the knowledge that willful, false statements and the like so made are

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patent issuing thereon.

Executed this 7th day of July 2004, at Richardson, Texas.

A. Dean Sherry

A. Dean Sherry, Ph.D.